MicroRNAs have emerged as key posttranscriptional regulators of gene expression during vertebrate development. We show that the miR-200 family plays a crucial role for the proper generation and survival of ventral neuronal populations in the murine midbrain/hindbrain region, including midbrain dopaminergic neurons, by directly targeting the pluripotency factor Sox2 and the cell-cycle regulator E2F3 in neural stem/progenitor cells. The lack of a negative regulation of Sox2 and E2F3 by miR-200 in conditional Dicer1 mutants (En1(+/Cre); Dicer1(flox/flox) mice) and after miR-200 knockdown in vitro leads to a strongly reduced cell-cycle exit and neuronal differentiation of ventral midbrain/hindbrain (vMH) neural progenitors, whereas the opposite effect is seen after miR-200 overexpression in primary vMH cells. Expression of miR-200 is in turn directly regulated by Sox2 and E2F3, thereby establishing a unilateral negative feedback loop required for the cell-cycle exit and neuronal differentiation of neural stem/progenitor cells. Our findings suggest that the posttranscriptional regulation of Sox2 and E2F3 by miR-200 family members might be a general mechanism to control the transition from a pluripotent/multipotent stem/progenitor cell to a postmitotic and more differentiated cell.